



# Comparison of anaerobic digestion strategies of nitrogen-rich substrates: Performance of anaerobic reactors and microbial community diversity



Elvira E. Ziganshina<sup>a</sup>, Emil M. Ibragimov<sup>a</sup>, Petr Y. Vankov<sup>a</sup>, Vasili A. Miluykov<sup>b</sup>, Ayrat M. Ziganshin<sup>a,\*</sup>

<sup>a</sup> Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan 420008, The Republic of Tatarstan, Russia

<sup>b</sup> Department of Technologies, A.E. Arbuzov Institute of Organic and Physical Chemistry, RAN, Kazan 420088, The Republic of Tatarstan, Russia

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## ABSTRACT

In the present study, the application of different operating strategies on performance of three continuous stirred tank reactors digesting chicken manure at mesophilic temperature and constant organic loading rate (OLR) of  $3.5 \text{ g}_{\text{VS}} \text{ L}^{-1} \text{ d}^{-1}$  was investigated. Control reactor (RC) and reactor (RH) with the decreasing hydraulic retention time (HRT) had the comparable specific biogas production (SBP) with maximum values of  $334\text{--}351 \text{ mL g}_{\text{VS}}^{-1} \text{ (added)}$  during days 84–93, while the SBP from reactor with zeolites (RZ) was higher and achieved  $426\text{--}432 \text{ mL g}_{\text{VS}}^{-1} \text{ (added)}$ . Attachments of microorganisms to zeolite particles as the operational environment, exchanged cations released from zeolites as well as lower total ammonium nitrogen (TAN) levels observed in RZ ( $6.2\text{--}6.3 \text{ g L}^{-1}$ ; days 71–93) compared to RC ( $6.6\text{--}6.9 \text{ g L}^{-1}$ ; days 71–93) resulted in a more effective process in RZ. Moreover, microbial community structure and dynamics were comprehensively characterized using Illumina sequencing, pyrosequencing and T-RFLP analysis of 16S rRNA genes. Methanogenic archaeal activity was additionally assessed by the expressed *mcrA* genes encoding the alpha subunit of methyl-CoM reductase. Within the major class *Clostridia*, *Caldicoprobacter*, *Alkaliphilus*, *Gallicola*, *Sporanaerobacter* and *Tepidimicrobium* spp. were the notable bacteria developed during operation of all tested reactors. Archaeal communities were dominated by methanogens belonging to the genus *Methanosarcina* followed by the genus *Methanoculleus* during the experimental period. Results of this study indicate that attachment of microorganisms to the zeolite particles as the operational environment might have led to the higher microbial activity at high ammonia concentrations.

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## 1. Introduction

A significant amount of animal manure is annually produced in Russia; however, in most cases, the uncontrolled storage of manures on agricultural lands results in the pollution of the atmosphere, soil and water resources. Such environmental pollution caused by various types of manures can be controlled by anaerobic digestion of agricultural organic wastes under both mesophilic and thermophilic conditions. Despite the fact that anaerobic conversion of residual biomass with the generation of biogas is not widely utilized in Russia, anaerobic digestion belongs to a more suitable method to treat various wastes resulting in minimization of their amount and obtaining energy in the form of energy-rich methane worldwide (Ye et al., 2013; Jia et al., 2015; Mazareli et al., 2016; Jensen et al., 2016; Li et al., 2016). Therefore, it is very important

to develop and put in practice new effective biotechnologies to solve the problems associated with the growing levels of organic wastes.

Anaerobic conversion of chicken manure into methane-rich biogas has become increasingly attractive in the last decades as a good choice to minimize wastes accumulation and recover bioenergy (Nie et al., 2015; Niu et al., 2015; Wu et al., 2016; Sun et al., 2016). However, chicken manure contains high nitrogen concentrations due to the presence of uric acid and undigested proteins. Their microbial decomposition results in the formation and accumulation of toxic ammonia in anaerobic systems. The total ammonia nitrogen (TAN) levels include ammonium nitrogen ( $\text{NH}_4\text{--N}$ ) and free ammonia nitrogen (FAN,  $\text{NH}_3\text{--N}$ ) concentrations. Free ammonia is pH, temperature and TAN dependent and its excess concentration has been widely considered as the major cause of microbial consortia inhibition over the course of anaerobic digestion of nitrogen-rich substrates. Ammonia inhibition processes as well as processes to overcome ammonia toxicity on anaerobic

\* Corresponding author.

E-mail address: [a.ziganshin06@fulbrightmail.org](mailto:a.ziganshin06@fulbrightmail.org) (A.M. Ziganshin).